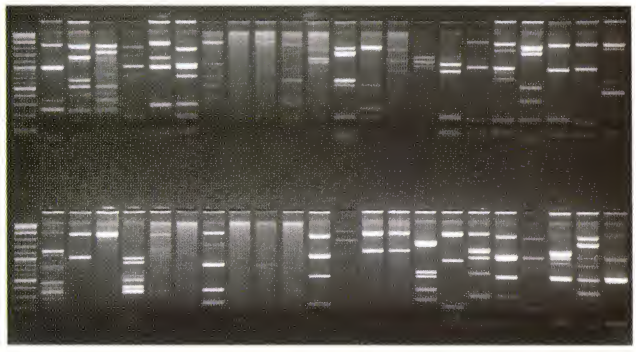
BIOTECHNOLOGICAL INTERVENTION FOR TACKLING THE LEAF CURL VIRUS DISEASE IN TOMATO

A virus infected tomato plant

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world and is a major vegetable in Bangladesh. Tomato yellow leaf curl virus (TYLCV) and Tomato leaf curl virus (ToLCV) are the two major geminiviruses which cause serious losses (up to 100%) to tomato production. Other major crops like okra, mungbean and cucurbits are also infected by related geminiviruses.

Proper identification and characterisation is a prerequisite for developing a control strategy against these viruses. Because of cross-reactivity, the commonly used ELISA technique cannot differentiate between closely related strains. Hence, precise PCR-based methods should be developed for characterisation and diagnosis of these viruses infecting tomatoes in Bangladesh. On the other hand, production of transgenic tomato plants is thought to be the only way to get sustainable resistance against these viruses. From a technical point of view, it is also important to focus on an understanding of the mechanisms on which engineered resistance is based. This information will allow efficient and reproducible transfer of resistance from model plants to crop plants. Genetic engineering remains an alternative and rapid method to transfer resistance genes to traditional cultivars, bypassing the long procedure of introgression and the appearance of undesired traits usually associated with it. Development of the genetic engineering tool and ongoing studies on geminiviruses will help to achieve stable resistance in geminivirus-susceptible crops. Successful transformation is also essential in basic and practical studies for tomato improvement. In addition, an efficient



Polymorphism in ToLCV samples collected from different parts of the country revealed by RCA-RFLP

and reliable regeneration protocol is prerequisite for such transformation work. Considering the above factors, Biotechnology Division of Bangladesh Agricultural Research Institute (BARI) has initiated a project in 2010 on "Molecular characterisation of Tomato Yellow Leaf Curl Virus (TYLCV) in Bangladesh and development of TYLCV resistant tomato using recombinant DNA technology", funded by PIU-BARC, NATP: Phase-1.

Over the last two years several experiments have been conducted to achieve the objectives of the project. A total of 46 isolates were analysed from samples collected from eight districts of the county and the presence of leaf curl virus has been confirmed in 44 using advanced molecular techniques. This group of viruses have mono- or bi-partite (A and B) genomes. Some strains have additional genomic particles in combination with the 'A part'. Among those 44 isolates, complete sequencing

Transformed tomato shoots growing on artificial medium

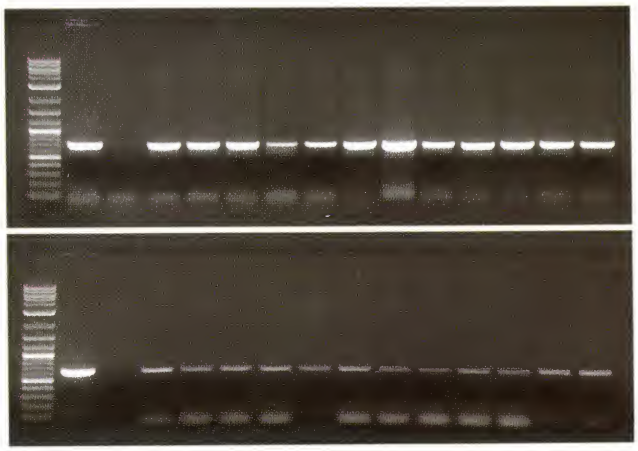
of 'A' genomes of 32 Tomato Leaf Curl Virus (ToLCV) isolates has been done which confirmed high level of polymorphism and the presence of several strains. However, further sequencing will be required on both the DNA strands for confirmation and standardisation of sequence information.

The sequencing data available so far were used for bioinformatics analyses and the phylogenetic relationships among various strains were determined. Presence of seven strains of Geminiviruses belonging to three major groups was confirmed in tomato crops from Bangladesh.

In preparation for developing virus resistant transgenic tomato varieties, genetic transformation protocol for a BARI tomato variety has been optimised using marker and reporter genes. Insertion of the transgenes has been confirmed in 12 transformed tomato plants by PCR using transgene-specific primers for *nptII* and *gus*, respectively.

Achievement

- Survey conducted in 8 districts and total 46 isolates of ToLCV collected
- 46 isolates analysed using RCA-RFLP method
- Complete 'A' genomes of 32 virus isolates sequenced.
- Two plasmid vectors with *nptII* and *gus* reporter genes constructed and used for optimization of genetic transformation protocol for a BARI released tomato variety.
- Insertion of the transgenes was confirmed by PCR in 12 transformed plants.



Confirmation of transgenes by PCR

Transgenic tomato growing in the greenhouse

Way forward:

The information generated so far, will help precise diagnosis of Geminiviruses infecting tomato and other crops in Bangladesh. These sequence information will be further used for constructing plasmid vectors to be used for transformation of tomato plants aiming at broad-spectrum resistance against Geminiviruses. The genetic transformation protocol developed using the marker and reporter genes will be used for the development of tomato plants expecting to have good resistance against leaf-curl virus diseases.

Lessons learned:

Previously it was thought that tomato leaf curl disease is caused by the Tomato Yellow Leaf Curl Virus (TYLCV) which was a symptom-based assumption. Our studies revealed that several strains of viruses belonging to the Geminivirus group are involved in causing tomato leaf curl diseases in Bangladesh. This is made possible by the use of advanced molecular diagnostic methods facilitated by the project. However, these complex results also mean that more time and further research will be required to achieve the ultimate objective of developing virus resistant tomato varieties.

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For details, please contact: **Dr. M A Yousuf Akhond**

Principal Scientific Officer, Biotechnology Division, BARI, Gazipur and Principal Investigator, "Molecular characterisation of Tomato Yellow Leaf Curl Virus (TYLCV) in Bangladesh and development of TYLCV resistant tomato using recombinant DNA technology" sub-project of PIU-BARC, NATP, Phase I. E-mail: *a_akhond@hotmail.com*